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Parallel detection of bacteria with culture methods and 16S *rRNA* gene analysis: Preliminary results from a prospective cohort study of prosthetic joint infections

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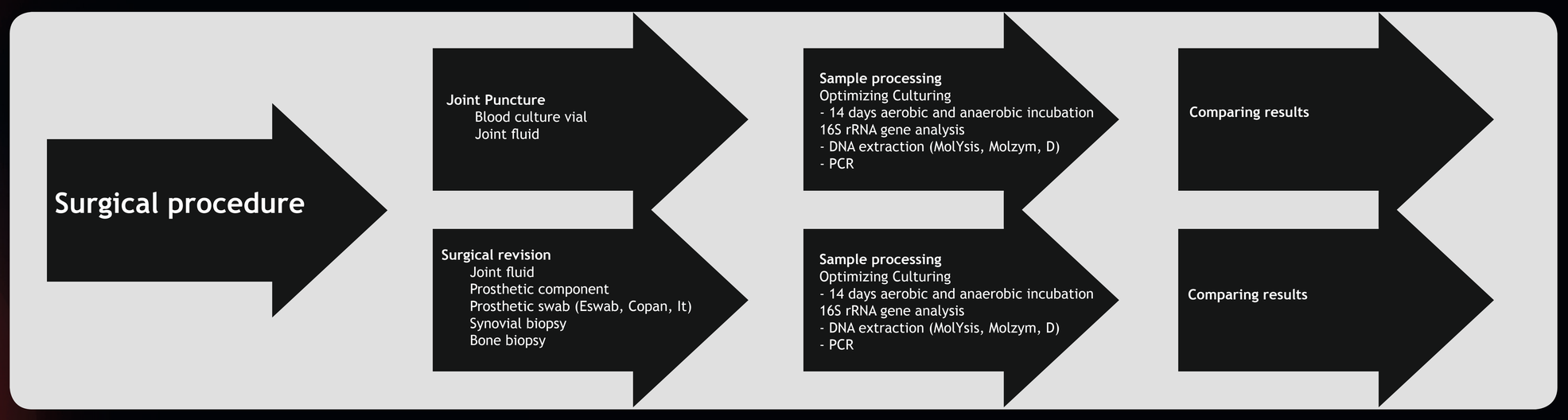
Introduction

'Artificial joints' have become indispensable treatment for osteoarthrosis. Approximately 15,000 primary arthroplastic procedures and 2,000 revisions are performed each year in Denmark (1). The number of patients with prosthetic implants will continue to grow mainly due to an aging population.
However, the implants can lead to serious complications, one of which is bacterial infection. The risk is estimated to be 0.5-2% for primary arthroplasties and even higher for revision surgeries. The infections are difficult to diagnose due to vague symptoms and a modest inflammatory response. Routine culture-based methods failure to demonstrate a bacterial agent in a significant proportion of cases. The failure may be due to inappropriate culture conditions, prior antibiotic treatment, fastidious or atypical organisms, and the biofilm mode of growth.
The successful diagnosis and treatment of prosthetic joint infections (PJIs) require a close cooperation between surgeons, radiologists, specialists in nuclear medicine, clinical microbiologists and molecular biologists. At Aalborg Hospital we have established a unique diagnostic algorithm within the framework of the PRIS Innovation project. In this presentation the objective was to compare positivity rates of extended cultures for the presence of bacteria with the positive rates of detection of 16S ***rRNA*** genes in parallel samples. We report preliminary results for the first 65 patients recruited consecutively December 2011 through June 2012.

M&M

The study comprised patients seeking medical attention with a painful hip or knee prosthesis. Twenty five had PJI according to the physician's judgement; the remaining 40 patients had an aseptic loosening (AL). Fifty four underwent surgical revision, seven had a joint puncture, and four had two procedures (joint puncture+revision or revision+revision).

The prosthetic components were processed by submerging into isotonic buffer (1XPBS) for sonication (40 kHz) and aliquots of the buffer were centrifuged to concentrate the bacterial content and used for further processing (2). Bacteriological cultures were cultivated on agar plates and in broth medium under aerobic and anaerobic conditions for 14 days. For full length 16S *rRNA* gene analysis DNA were extracted and screened by endpoint PCR.



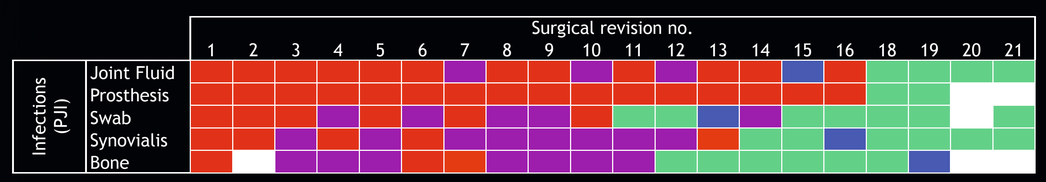
Flow diagram: Joint puncture; 3 samples were obtained, 2 for culture (1 aerobic blood culture vial and 1 for bacterial culturing) and 1 for 16S rRNA gene analysis. During revision up to 15 samples were obtained; 5 for 16S rRNA analysis, 5 for bacterial culturing and five for other purposes. Prior to this study, bone biopsies, prosthetic components, and biofilm swabs were not available for microbiological diagnosis of PJI.

Results

Surgical revisions (table). In the PJI group (n=18), a total of 74 samples were positive by culture and 58 by 16S *rRNA* gene analysis. We observed a high level of concordance between culture-based and molecular methods with specimens of sonication fluid and synovial fluid whereas results were less consistent with synovial biopsies, bone biopsies and biofilm swabs (table 1). The highest numbers of positive specimens were obtained with sonication fluid, followed by synovial fluid and tissue biopsies. In the AL group of patients (n= 40) one sample was positive only by culture and 14 by 16S *rRNA* gene analysis.
Joint puncture. One sample of synovial fluid for one patient was positive both by culture and 16S *rRNA* gene analysis, and samples from six other patients were negative by both culture and 16S *rRNA* gene analysis.

Discussion

Prosthesis components show promise as a new sample type for the diagnosis of PJI. Using the established sonication procedure (2,3,4) and optimized culture methods we obtained agreement with 16S rRNA gene analysis in 57 procedures in 56 patients. Sonication has also previously been shown to be valuable for culturing of prosthetic components and to increase rates of bacterial growth (5).
At the current time we interpret the positive results in the AL group as spurious, but a conclusion cannot be reached before sequencing has been undertaken.
Our results are promising for in depth exploration of the microbiology of prosthetic joint infections. Parallel sampling for culture and 16S rRNA gene analysis allow an extensive evaluation of the diagnostic accuracy of different sample types and methods. A high concordance between clinical assessment and results obtained by optimized culture methods is indicated in several reports (2,3,4) and with the large patient series in the PRIS project these methods can be thoroughly tested against each other with different sample types.
We expect that the results from the ongoing PRIS project can contribute to the rational foundation for diagnosis of suspected prosthetic joint infections.



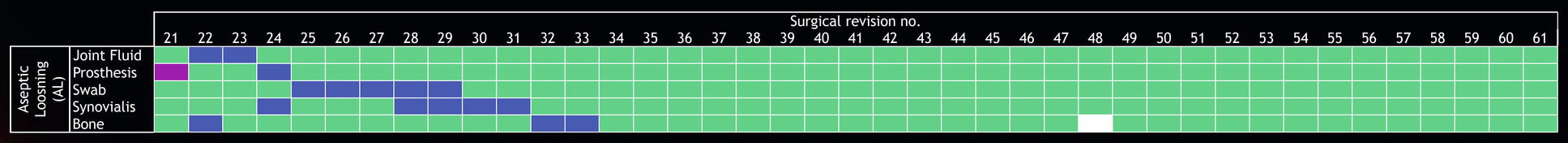


Table 1:
Concordance between 16S rRNA gene anlysis (PCR) and optimized culture methods. Red: PCR+Culture positive. Blue: PCR positive. Purple: Culture positive. Green PCR+Culture negative. White: No sample available. Two patients have two sugical procedures in the Infection Group (PJI).

Acknowledgement
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www.joint-prosthesis-infection-pain.dk

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